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Efficacy and residues of phloxine B and uranine for the suppression of Mediterranean fruit fly in coffee fields

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Abstract: The field efficacy of a bait containing phloxine B, uranine and Provesta 621 protein was tested against Mediterranean fruit fly (*Ceratitis capitata*; Medfly) by aerial and ground spraying in about 84 ha of coffee fields in Kauai, Hawaii, USA. Concurrently, soil and crop samples were collected from the aerially sprayed field and its unsprayed control field for residue studies. Efficacy of the sprays was assessed through trapping with both protein-baited and trimedlure-baited traps and through the infestation level of coffee cherries collected at least three-quarters ripe. The *C capitata* population was low at the start of the aerial and ground spray studies, but dramatically increased in the control fields. This increase coincided with initial ripening of coffee cherries. During times of peak population levels, *C capitata* populations were reduced by more than 91% in the ground-sprayed field and 99% in the aerial-sprayed field, relative to the populations in their respective control fields and based on protein-baited trap catches. Results of residue analyses indicated that uranine dissipated quickly compared with phloxine B on coffee and soil. Coffee samples collected at pre-spray periods had phloxine B residues of 7.2–25.5 ng g^{-1} on berries. Phloxine B concentrations were much higher on coffee leaves (163–1120 ng g^{-1}). Lower concentrations of the dye were found from coffee samples collected during rainy days. Average phloxine B concentrations immediately after spraying were 56 and 2840 ng g^{-1} in coffee berries and leaves, respectively. Dissipation of phloxine B on berries was fast, with a half-life ($t_{1/2}$) of 3 days. Dissipation of phloxine B on leaves was fitted to two linear phases: the initial (0–4 days) with a shorter $t_{1/2}$ of 3 days and the later phase (4–28 days) with a longer $t_{1/2}$ of 15 days. Average concentrations of phloxine B in the top soil ranged from 50 to 590 ng g^{-1} at pre-spray. Phloxine B initial concentration (770 ng g^{-1}) reached a plateau immediately after the last spraying, but showed a steady decline over time with $t_{1/2}$ of 16 days. Fast dissipation of the dyes in the field indicates that these chemicals may be environmentally compatible and therefore a promising alternative for fruit fly control.

Keywords: phloxine B; uranine; coffee; insecticide; dissipation; fruit fly

1 INTRODUCTION

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann), infests more than 300 varieties of fruit and vegetable.[1] This wide host range poses a threat to the agricultural industry of any region in which this fly species becomes established. The organophosphate malathion mixed with a protein bait has traditionally been used to control *C capitata* and other tephritid fruit fly populations. However, concerns over environmental and human health hazards attributable to malathion and its oxidative product have prompted screening of environmentally compatible alternatives. The xanthene dyes phloxine B (D&C red dye No 28) and uranine (D&C yellow dye No 8) (Fig 1) have been used for decades to color drugs and cosmetics, and are considered safe for human use.[2] These photoactive dyes are promising, more environmentally friendly, alternative toxicants to malathion in bait sprays.[3–5] Uranine, though apparently not pesticidal on its own, has been found to synergize the toxicity of a xanthene dye (Rose Bengal) to mosquito *Aedes triseriatus* (Say) larvae, possibly by extending the range of light wavelengths from which energy can be absorbed and utilized to activate the Rose Bengal.[6] Because of this potential for synergism, early tests of efficacy of photoactive dyes against *C capitata* (including the

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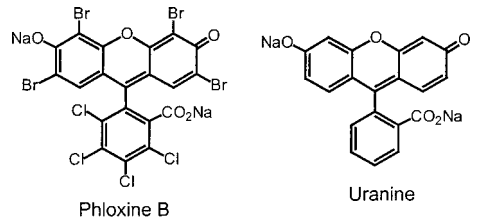

Figure 1. Structures of phloxine B (D&C red dye no 28) and uranine (D&C yellow dye no 8).

present study) included both phloxine B and uranine. Subsequent research has shown that uranine does not synergize the activity of phloxine B for *C capitata* at light intensities varying from 17 to 260 $\mu E\,m^{-2}\,s^{-1}$ (1000–16 000 lux), which includes lower light levels such as might be encountered within the foliage of host plants (McQuate GT, unpublished results). Subsequent field spray trials, with uranine dropped out of the bait spray, have shown good suppression of tephritid fruit fly populations.[7–9] Unlike malathion, these dyes do not kill flies on contact but must be ingested and subsequently exposed to light to cause mortality. Consequently, non-target insects which do not consume the bait are unlikely to be killed by the bait spray. Several laboratory studies have documented the resulting reduced toxicity to non-target organisms. Phloxine B and uranine, both alone and when mixed with protein baits, had no measurable contact toxicity to six species of beneficial insect, and the toxicity of phloxine B and uranine in protein baits was significantly less for all six species than that of protein baits incorporating malathion.[10] In another study, a mixture of phloxine B and uranine or phloxine B alone in a protein bait had minimal toxicity to a *C capitata* parasitoid, *Fopius arisanus*, relative to its effect on *C capitata* itself.[11] Malathion, however, did not exhibit selectivity, both *C capitata* and its parasitoid showing high levels of mortality when exposed to a protein bait mixed with malathion. A possible mechanism of the photodynamic action of phloxine B involves formation of singlet oxygen which reacts with target molecules.[12] The key to successful use of xanthene dyes for fruit fly control lies in the development of selective baits so that the risk to non-targets is reduced and toxicity on target species is enhanced.

In Hawaii, coffee is commonly found to be infested by *C capitata*. Increase in coffee acreage on the island of Kauai led to seasonally high populations of the pest,[13] providing a cropping environment well suited to testing the efficacy of xanthene dye–protein bait sprays for tephritid fruit fly population suppression. An initial field trial involving ten weekly aerial sprays on a 51-ha semi-isolated coffee field resulted in significant reductions in *C capitata* population levels (McQuate GT, unpublished results). Residue data from this initial field trial were previously reported.[14] The present work is a field test of longer duration, designed to determine simultaneously the field efficacy of a protein bait solution incorporating phloxine B and uranine, and the dissipation of the dyes in coffee and soil following 22 (aerial) or 23 (ground) weekly sprays.

2 EXPERIMENTAL

2.1 Field trial location and spray treatment

2.1.1 Ground-sprayed field

A weekly ground spray of a xanthene dye–protein bait solution was applied to 20.2 ha of a 32.4-ha coffee field in Koloa on the island of Kauai, Hawaii, USA. The formulation used and the quantity of solution applied per hectare are presented in Table 1. Phloxine B (94% purity) and uranine (77.1%) were obtained from Hilton Davis (Cincinnati, OH, USA). Ground spraying was done with Tee Jet 5500-X1 and 5500-X2 (Spraying Systems Co, Wheaton, IL, USA) cone jet spray nozzles mounted on an all-terrain vehicle. Two bands of the dye-bait spray were projected onto the underside of the foliage using a pump that maintained a force of 3.5–4.2 $kg\,cm^{-2}$. Weekly ground spraying began on 7 August 1996 and continued until 4 February 1997 for a total of 23 sprays. By the last spray, few ripe coffee berries remained on the plants.

2.1.2 Aerially sprayed field

Aerial spraying was done with a helicopter applying an ultra-low-volume spray. The formulation used and the quantity of solution applied per hectare are presented in Table 1. Weekly aerial spray applications over 42.9 ha of a 51.0-ha semi-isolated coffee field in Kipu, Kauai were begun on 22 August 1996, and were continued through 30 January 1997 for a total of 22 sprays. The field temperature fluctuated between 20.5 and 21.5 °C during the experimental period. Rainfall data during this period were recorded by a weather station in Kipu, Kauai (see Fig 4A).

2.2 *Ceratitis capitata* population assessment for efficacy determination

2.2.1 Trap catches—ground-sprayed field

Ten trimedlure (TML)-baited traps and ten McPhail traps baited with protein bait solution were placed

	Aerial spray		*Ground spray*	
Ingredient	*Formulation ($g\,kg^{-1}$)*	*Rate ($g\,ha^{-1}$)*	*Formulation ($g\,kg^{-1}$)*	*Rate ($g\,ha^{-1}$)*
Phloxine B	6.8	27.3	2.1	102.3
Uranine	3.2	11.9	1.0	46.2
Provesta 621	300	1180	25	1190
Water	690	2710	971.9	46690

Table 1. Concentrations of ingredients in protein bait spray formulations and application rates for both ground and aerially applied spray trials

within the treated portion of the 32.4-ha coffee field (0.5 trap of each type per ha) and 10 traps of each type were placed in a 35.7-ha unsprayed control field (0.28 traps per ha). Each trap held a protein bait solution which consisted of 10% Provesta 621 [an autolyzed yeast extract obtained from Integrated Ingredients (Bartlesville, OK, USA)], 3% borax and 87% water. Traps were placed in the fields before the first spray and were serviced weekly until 4 weeks after the last spray.

2.2.2 Trap catches—aerially sprayed field

Fifteen TML-baited traps and 15 McPhail traps baited with protein bait solution, as described in Section 2.2.1, were placed within the treated portion of the 51.0 ha coffee field (0.35 traps of each type per ha) and 10 traps of each type were placed in a 39.6 ha unsprayed control field (0.25 traps per ha). Traps were placed in the fields before the first spray and were serviced weekly until 4 weeks after the last spray.

2.2.3 Fruit infestation

Coffee berries, at least three-quarters ripe, were collected weekly from five 3.0-m sections of coffee row from both the control and the aerially sprayed treatment fields from 26 August 1996 through 17 February 1997, two and a half weeks after the last spray. Similarly, weekly collections were made from both the control and the ground-sprayed treatment fields from 26 August 1996 through 26 January 1997. Further berry collections were not possible because there were no more ripe cherries in the ground-sprayed field. Collected coffee berries were held for assessment of the infestation. Infestation level was summarized as average number of fruit fly adults and unemerged pupae per 100 g of coffee berries.

2.3 Sampling procedures for soil, coffee berries and leaves

The 42.9-ha coffee treatment field was divided into four major sites which were further divided into four sub-sites to facilitate sampling. Soil samples (about 1 kg) were randomly collected at three soil layers (0–5, 5–10 and 10–15 cm) from each sampling site. For a total of 22 spray applications, samples were collected from 12 occasions, before the 1st, 3rd, 5th, 7th, 8th, 10th, 12th, 15th, 17th, 19th, 21st and 22nd spraying, to determine the deposition rate of the dye in soil. Soil samples were also collected immediately after the 22nd spraying and 1, 4, 11, 19, 28 and 45 days after the 22nd spraying for the dissipation study. Concurrent with soil collection, about 1 kg of coffee berries (at least three-quarters ripe) and 0.5 kg of leaves were also randomly collected from coffee trees at each site for 12 occasions until the 22nd spraying. In addition, coffee berries and leaves were also collected immediately after the 22nd spraying, and at 1, 4, 11, 19, 28 and 45 days, at the same time as the soil sampling. The samples were transported by air to the laboratory and were kept frozen at −15 °C until extracted.

2.4 Analytical procedure for phloxine B and uranine

2.4.1 Soil

The frozen soil samples were brought to room temperature, air-dried, sieved (20-mesh) and stored in mason jars in the dark at room temperature. The moisture content of each soil sample (20 g) was determined. The dyes were extracted from soil (control or treated) by supercritical fluid extraction (SFE) using carbon dioxide, and analyzed by HPLC as previously described.[15]

2.4.2 Coffee berries

Frozen coffee berries (0.5 kg) were ground with dry ice at 1:1 ratio, transferred to mason jars and allowed to dissipate in a freezer overnight (−10 °C). A portion of the ground berries (20 g) was oven-dried for moisture determination. A 25-g sample of coffee berries (control or treated) was macerated in a Sorvall Omni mixer for 5 min with methanol + acetonitrile + *n*-butylamine (1 + 1 + 0.05 by volume; 300 ml). The extract was subjected to cation solid phase extraction (SPE) clean-up using an amino column and analyzed by HPLC following the published procedure.[16,17]

2.4.3 Coffee leaves

About 100 g of coffee leaves were cut into pieces of about 1 cm^2 area and 20 g of the sample was oven-dried for moisture determination. Fifty grams of the chopped leaves (control or treated) were transferred into a 1-quart mason jar and soaked in methanol + acetonitrile (1 + 1 by volume; 300 ml) for 1 h. The mixture was homogenized in a Sorvall mixer at medium speed for 5 min and vacuum filtered through a fiber-glass filter in a Buchner funnel. An aliquot (100 ml) of the extracts was transferred into a 250-ml separatory funnel and defatted with hexane (2 × 50 ml). The hexane layer was discarded and the remaining extract subjected to amino-SPE column clean-up. The SPE column (1 g) was first washed with methanol, then activated with methanol + 0.05 M hydrochloric acid (9 + 1 by volume) before the sample was applied. Hexane, acetone and then methanol (7 ml each) were passed through the column to remove interference. The analytes were finally eluted with methanol + 0.1 M sodium hydroxide (9 + 1 by volume; 15 ml), concentrated to 2 ml and filtered in Gelman acrodisc for HPLC analysis. Phloxine B and uranine were analyzed by HPLC as previously reported.[16] The dye residues were reported on a dry weight basis for all the samples.

2.4.4 Recovery study

A measured amount of phloxine B and uranine in methanol (20 μg ml^{-1}) was used to spike the control soil and coffee samples for evaluating the efficiency of the analytical procedures used. The method detection limits and the mean recoveries of these analytes in soil and coffee samples are tabulated in Table 2. The mean recoveries of phloxine B were 87 (±10)% from fortified

Sample	Phloxine B		Uranine	
	Mean recovery[a] *(%) (CV)*[b]	*MDL*[c] *(ng g^{-1})*	*Mean recovery*[a] *(%) (CV)*[b]	*MDL*[c] *(ng g^{-1})*
Soil[d]	87 (10)	7–10	83 (11)	20–30
Coffee berries[e]	82 (5)	2–4	81 (6)	15–17
Coffee leaves[f]	85 (10)	20–30	83 (11)	20–30

[a] Based on spiked control samples and computed on dry basis. No dye was found in control samples.
[b] *CV* = Coefficient of variation.
[c] *MDL* = Method detection limit. Signal-to-noise ratio is 3:1.
[d] Mean of all recoveries of the analytes added at 0.063–2.5 μg g^{-1} soil.
[e] Mean of all recoveries of the analytes added at 0.005–1.00 μg g^{-1} coffee berries.
[f] Mean of all recoveries of the analytes added at 0.063–2.5 μg g^{-1} coffee leaves.

Table 2. Recovery of phloxine B and uranine from soil and coffee

soil, 82 (±5)% from coffee berries and 85 (±10)% from leaves. Uranine recoveries were 83 (±11)% in soil, 81 (±6)% from coffee berries and 83 (±11)% from leaves.

3 RESULTS AND DISCUSSION

3.1 Field control efficacy

3.1.1 Ground-sprayed field trap catch

At the start of the field trial by ground spraying, the *C capitata* population was low (Fig 2) but increased dramatically in the control field. This increase coincided with initial ripening of coffee berries. There was, however, no comparable increase in the *C capitata* population in the treatment field. From the first trap catch after the first spray (12 August) until the *C capitata* population level declined in the control field (18 November), the fly reduction in the sprayed field relative to the control field averaged 72% and 91% based on the number of fruit flies caught in TML-baited traps and protein-baited traps, respectively (Figs 2A and B). The fly population in the sprayed field increased some time in early November at the time when a rainy period caused spray cancellations resulting in a 16-day gap between sprays. The *C capitata* population level declined again once the weekly spraying was resumed, but also declined in the control field because of a coffee harvest on 26 October. From the next trap catch after the *C capitata* population level declined in the control field (25 November) until the week after the last spray (10 February) the fly reduction in the sprayed field relative to the control field averaged 78% and 90% based on the number of fruit flies caught in TML-baited traps and protein-baited traps, respectively.

3.1.2 Aerially sprayed field trap catch

The same population trend was observed in the control field associated with the field which received the dye applied by aerial spraying. The *C capitata* population was low at the start of the experiment but increased dramatically in the control field (Figs 3A and B). From the first trap catch after the first spray (26 August) until the level of the *C capitata* population declined in the control field (29 October), the fly reduction in the sprayed field relative to the control field averaged 98% and 99% based on the number of fruit flies caught in TML-baited traps and protein-baited traps, respectively (Figs 3A and B). The population reductions in the treatment field relative to the control field were greater than 99% based on numbers of the flies caught in both TML-baited and protein-baited traps during the early weeks of spray (Figs 3A and B). A decline of the *C capitata* population in the control field, as seen in both TML-baited and protein-

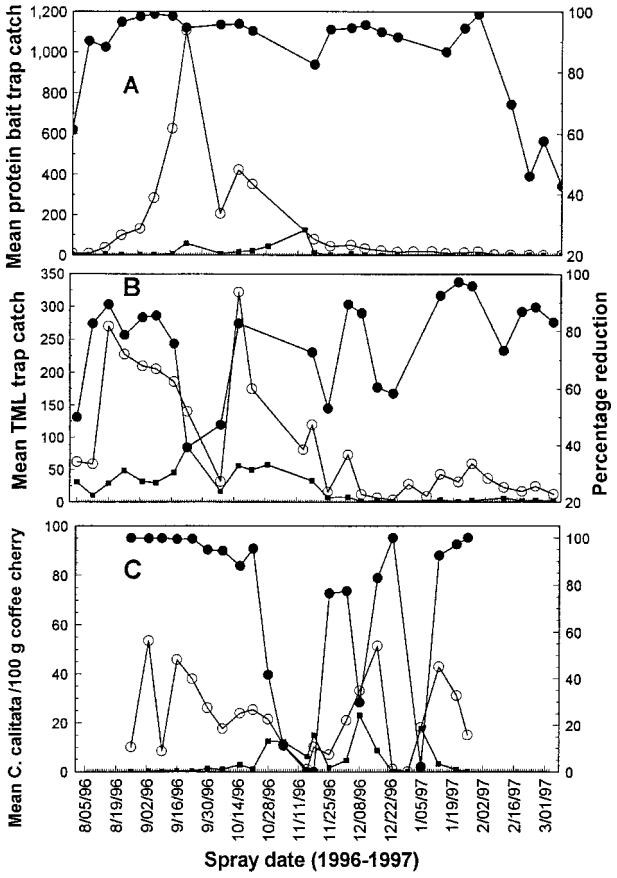

Figure 2. Average catch of *Ceratatis capitata* in (A) protein-baited traps, (B) in trimedlure-baited traps, and (C) average infestation of *Ceratatis capitata* per 100 g of coffee berries in (■) a field ground sprayed with a protein bait containing phloxine B and uranine and (○) in an unsprayed control field. (●) Average percentage reduction of catch (A, B) or infestation rate (C) in the sprayed field relative to the control field.

baited traps, followed the harvest of all coffee cherries there (1 November 1996), with the result that the *C capitata* population level in the control field dropped to a level similar to that in the sprayed field. From the next trap catch after the *C capitata* population declined in the control field (18 November) until the week after the last spray (3 February), the fly reduction in the sprayed field relative to the control field averaged 60% and 84%, based on the number of fruit flies caught in TML-baited traps and protein-baited traps, respectively.

3.1.3 Ground-sprayed field berry infestation

As coffee berries ripened there was considerable increase in infestation rate in the control field, while there was a continued low infestation in the sprayed field. The infestation rate was reduced more than 99% during the early weeks of the spray program (Fig 2C).

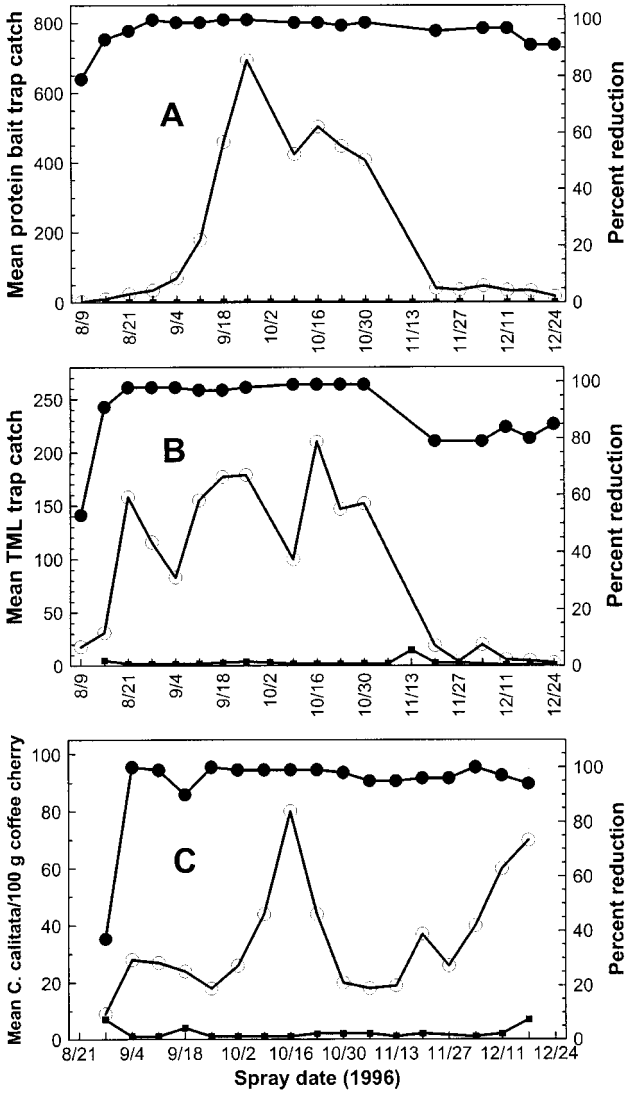

Figure 3. Average catch of *Ceratatis capitata* in (A) protein-baited traps, (B) in trimedlure-baited traps, and (C) average infestation by *Ceratatis capitata* per 100 g of coffee berries in (■) a field aerially sprayed with a protein bait containing phloxine B and uranine and (○) an unsprayed control field. (●) Average percentage reduction of catch (A, B) or infestation rate (C) in the sprayed field relative to the control field.

Coffee berry infestation increased in the sprayed field at the time of the temporary suspension of spraying, and tended to fluctuate from week to week after that because harvesting of ripe coffee berries left fewer fruits available for oviposition and fewer fruits for sample collections.

3.1.4 Aerially sprayed field berry infestation

The berry infestation data showed that there was a considerable reservoir of *C capitata* in infested coffee cherries in the control field throughout the field experiment (Fig 3C). All coffee cherries collected from September to the end of December from the sprayed field showed at least a 90% reduction in fly infestation (Fig 3C). Reduction in the percentage infestation of berries in the sprayed field varied toward the end of the trial, as in the ground-sprayed trial, as the number of ripe coffee cherries in the field decreased. Overall, the reduced infestation rates in the sprayed field were consistent with the reduced fly catches, and thus showed the effectiveness of the formulated dye for the suppression of the *C capitata* population.

3.2 Dissipation of xanthene dyes in coffee berries and leaves

Phloxine B and uranine were not found in coffee berries and leaves collected before the 1st, 3rd and 5th spray applications. Deposition of phloxine B only was first detected in both berry and leaf samples collected before the 7th spraying. Phloxine B residues at pre-sprays ranged from 0.007 to 0.026 $\mu g\,g^{-1}$ in coffee berries (Fig 4B) and between 0.16 and 1.12 $\mu g\,g^{-1}$ in leaves (Fig 4C) until before the last spray (22nd). Low concentrations of phloxine B in the cherries and leaves before the 8th, 10th, 12th, 17th, 21st and 22nd spraying were probably attributable to a rainfall effect,

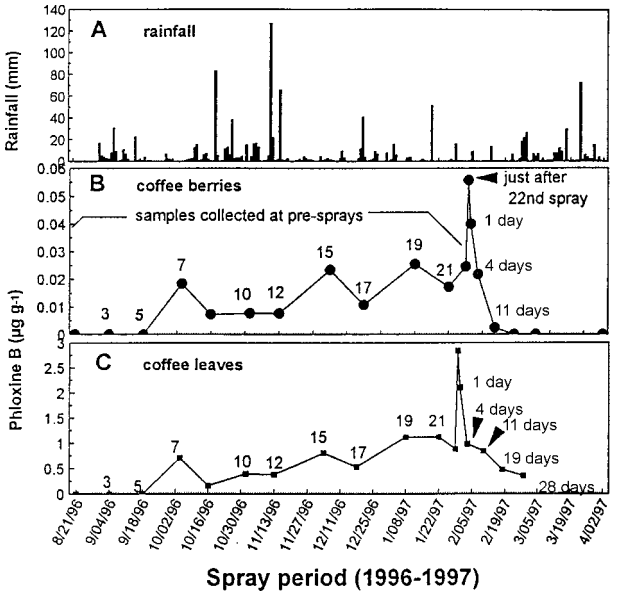

Figure 4. (A) Rainfall, (B) phloxine B residue on coffee berries and (C) on coffee leaves in a Kipu, Kauai coffee field which received weekly aerial sprays of a protein bait spray containing phloxine B and uranine.

as shown in Fig 4A. However, phloxine B concentrations detected in the leaves were over a hundred times higher than those in the berries, which is probably due to the difference in surface area between berries and leaves for the same unit weight of sample. In addition, the dye attached to lower leaf surfaces may not be washed off by rainfall as easily as that on coffee berries which have a rounded surface.

The dye residues reached their highest level immediately after the 22nd spray. In coffee berries, uranine (data not shown) was detected in berries at a level of $22\,ng\,g^{-1}$, but rapidly dissipated after one day to $15\,ng\,g^{-1}$, which was the limit of detection for this compound (Table 2). A higher concentration of uranine was found in coffee leaves ($0.24\,\mu g\,g^{-1}$) immediately after spraying, but it decreased to 0.13 and $0.02\,\mu g\,g^{-1}$ after 1 and 4 days, respectively. The half-life ($t_{1/2}$) of uranine in coffee berries and leaves is approximately 2 days.

Average initial concentrations of phloxine B on the leaves ($2.84\,\mu g\,g^{-1}$) were much higher than those on berries ($55.7\,ng\,g^{-1}$) immediately after the 22nd spraying (Figs 4B and C). For leaves, better correlation coefficients were obtained when the dissipation was fitted to two linear phases: the initial (0–4 days) with a shorter half-life (2.6 days) and the later phase (4–28 days) with a longer $t_{1/2}$ of 15.4 days. Phloxine B was found on berries until 11 days only, with a $t_{1/2}$ of 3.1 days. These data indicated a similar persistence of phloxine B in the leaves and fruits between 0 and 4 days, but phloxine B slowly dissipated from the leaves after 4 days.

3.3 Field soil dissipation of xanthene dyes

Phloxine B deposition in the top soil ranged from 0.05 to $0.59\,\mu g\,g^{-1}$ at pre-spraying periods which were approximately 5–6 days after the previous spray (Fig 5). Uranine was not found in any of the pre-spray soil samples. However, uranine was found in top soil immediately after spraying at a concentration of $0.33\,(\pm 0.07)\,\mu g\,g^{-1}$ and rapidly dissipated to $0.05\,(\pm 0.02)\,\mu g\,g^{-1}$ after 4 days and was no longer detected after 9 days. The $t_{1/2}$ of uranine in soil was estimated as 1.5 days.

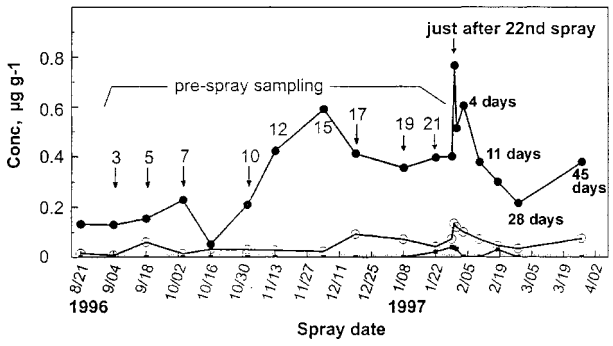

Figure 5. Dissipation of phloxine B in three soil layers: [●] 0–5, [○] 5–10 and [■] 10–15cm, of a Kipu, Kauai coffee field which received weekly aerial sprays of a protein bait containing phloxine B and uranine.

Dissipation of phloxine B in soil was monitored for 45 days after the 22nd spraying. The average concentration of phloxine B ($0.77\,\mu g\,g^{-1}$) reached a plateau immediately after spray and gradually declined to $0.21\,\mu g\,g^{-1}$ by the end of 28 days. The $t_{1/2}$ of phloxine B in the soil was 16.2 days. Surprisingly, an increase in phloxine B concentration ($0.38\,\mu g\,g^{-1}$) was observed in the soil samples collected after 45 days, which may be due to wash-off of the dye from leaves by frequent rainfall between day 28 and 45.

Low concentrations of phloxine B were also detected in the 5–10 and 10–15-cm layers of soil. Phloxine B concentration ranged from 8.1 to 72.7 ng g^{-1} in the 5–10-cm soil layer. Phloxine B was detected at the highest concentration of $39.9\,ng\,g^{-1}$ in the 10–15-cm layer at the latter part of the spraying. No uranine was detected below the top soil layer.

4 CONCLUSION

The results of this study show the effectiveness of xanthene dyes for the suppression of *C capitata* populations, whether applied through aerial or ground sprays. The first nine weekly aerial sprays of a protein bait containing 1% phloxine B and uranine gave over 99% reduction in population levels of *C capitata* relative to control field population levels during times of peak populations, based on protein-baited trap catches. Based on TML-baited traps, over 98% population reduction was observed over the same time period. Fourteen weekly ground sprays of 1% phloxine B and uranine in aqueous solution of 2.48% protein hydrolysate also led to over 91% reduction in *C capitata* population during times of peak populations, based on protein-baited trap catches. On the basis of the TML-baited traps, over 71% population reduction was observed over the same time period.

Dissipation of xanthene dye residues following spraying indicated the relatively higher persistence of phloxine B in soil, coffee leaves and berries compared with uranine. Uranine dissipated fast in the top soil and crop samples, with a $t_{1/2}$ of less than 3 days. It was not found in any of the sub-soil layers. Dissipation of phloxine B in leaves occurred in two stages: initially fast at 0–4 days with a $t_{1/2}$ of 2.6 days, then slower at 4–28 days with a $t_{1/2}$ of 15.4 days. In coffee berries, phloxine B dissipated fast with a $t_{1/2}$ of 3.1 days but stayed longer in top soil with a $t_{1/2}$ of 16.2 days. The $t_{1/2}$ values of phloxine B in coffee berries and soil are higher than those determined during the first field trial as reported earlier.[10] The current field test involved 22 weekly spray applications, compared with just 10 weekly sprays during the first field test. Higher deposition of the dyes in soil is expected because higher average weekly precipitation (40.7 mm) was recorded during this field test than in the first field trial, which had an average weekly rainfall of only 28.1 mm. Overall, the result of this field test has shown that phloxine B and uranine are an efficacious alternative for malathion, the currently used toxicant for

fruit fly control. The advantages of these dyes include low mammalian toxicity, lessened effects on non-target organisms, and non-persistence in the environment.

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